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Extension of Recombinant Human RANTES by the Retention of the Initiating Methionine Produces a Potent Antagonist*

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Extension of recombinant human RANTES by a single residue at the amino terminus is sufficient to produce a potent and selective antagonist. RANTES is a proinflammatory cytokine that promotes cell accumulation and activation in chronic inflammatory diseases. When mature RANTES was expressed heterologously in *Escherichia coli*, the amino-terminal initiating methionine was not removed by the endogenous amino peptidases. This methionylated protein was fully folded but completely inactive in RANTES bioassays of calcium mobilization and chemotaxis of the promonocytic cell line THP-1. However, when assayed as an antagonist of both RANTES and macrophage inflammatory polypeptide-1α (MIP-1α) in these assays, the methionylated RANTES (Met-RANTES) inhibited the actions of both chemokines. T cell chemotaxis was similarly inhibited. The antagonistic effect was selective since Met-RANTES had no effect on interleukin-8- or monocyte chemotractant protein-1-induced responses in these cells. Met-RANTES can compete with both [^{125}I]RANTES and [^{125}I]MIP-1α binding to THP-1 cells or to stably transfected HEK cells recombinantly expressing their common receptor, CC-CKR-1. These data show that the integrity of the amino terminus of RANTES is crucial to receptor binding and cellular activation.

RANTES is a member of a large family of cytokines, known as chemokines, which have the ability to recruit and activate a wide variety of proinflammatory cell types (1). They are small polypeptides of 8–10 kDa and have been further classified into CXC or CC chemokines based on the spacings of the cysteine residues proximal to the amino terminus. CXC chemokines primarily activate neutrophils, whereas CC chemokines have effects on several leucocyte cell types. RANTES is a CC chemokine, and *in vitro* it can produce chemotaxis and activation of monocytes, eosinophils, and T cells, particularly $CD4^+CD45RO^+$ (memory) T cells (2), but not neutrophils. These results imply a role for RANTES in diseases such as allergen induced late phase skin reactions or in allergic asthma. This hypothesis is strengthened by the fact that large amounts of RANTES are found in nasal polyp tissues, which are rich in infiltrating eosinophils (3). In addition, injection of RANTES into dog skin has been shown to induce a large eosinophilic infiltrate *in vivo* (4), and migration of human T lymphocytes was observed on injection of human RANTES into a human/severe combined immune deficiency mouse model (5).

MIP-1α shares an overlapping cell-type specificity with RANTES *in vitro* (6, 7) and has been shown to elicit an inflammatory response mediated through mast cell degranulation *in vivo* (8). A common receptor for these two CC chemokines has been cloned (9, 10) and is a member of the seven transmembrane G-protein linked receptor family. Recombinant expression of the receptor has shown that it can transduce a functional response on stimulation by both chemokines.

We report the purification of human RANTES expressed heterologously in *Escherichia coli*. In this system, the protein retains its initiating methionine residue, which renders it inactive as an agonist, while enabling it to antagonize effects induced both by RANTES and MIP-1α. It is able to compete for binding of both the radiolabeled ligands on THP-1 cells and to the recombinant RANTES/MIP-1α receptor with high affinity.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—The cDNA coding for RANTES was cloned from a human peripheral blood monocyte λGT11 cDNA library by PCR using primers based on the published sequence (2). The resultant PCR product was subcloned into the *E. coli* expression vector pT7-7 (11) and transformed into *E. coli* strain BL21(DE3). High level expression of T7 polymerase and RANTES was induced by addition of isopropyl-1-thio-β-D-galactopyranoside to the medium.

The protein was purified from inclusion bodies by gel filtration on a Sephacryl S-300 HR column equilibrated in 0.1 M Tris-HCl buffer, pH 8.0, containing 6 M guanidine HCl and 1 mM dithiothreitol. Renaturation was carried out by a 20-fold dilution into 0.1 M Tris-HCl buffer, pH 8.0, containing 1 mM oxidized glutathione and 0.1 mM reduced glutathione and stirring overnight at 4 °C. The renatured protein was concentrated by adjusting the pH to 4.5 with acetic acid, and loading onto a HiLoad SP 26/10 column equilibrated in 50 mM sodium acetate buffer, pH 4.5. The protein was eluted with a gradient of 0.6–2.0 M NaCl in the same buffer. The Met-RANTES[1]-containing fractions were dialyzed extensively against 1% acetic acid, and then against 0.1% trifluoroacetic acid and lyophilized. Recombinant full-length human RANTES and MIP-1α were expressed, purified, and renatured from *E. coli* (12).

Analytical Methods—SDS-PAGE was carried out on 4–20% acrylamide mini-gels (Novex) according to the manufacturer's instructions, and the proteins were visualized by staining with Coomassie Brilliant Blue R-250. Purified RANTES was quantified by the extinction coefficient of $A^{0.1\%}_{1\ cm}$ = 1.6 at 280 nm calculated from the amino acid sequence. Amino acid analysis was carried out by gas phase hydrolysis under vacuum in a nitrogen atmosphere for 24 h in 6 M HCl containing 1 mg/ml phenol at 112 °C. The resultant amino acids were quantified using a Beckman 6300 system using norleucine as an internal standard. Protein sequence was obtained with a model 477A protein sequencer (Applied Biosystems) using on line quantification of amino acid phenylthiohydantoin derivatives with a model 120A phenylthiohydantoin-derivative analyzer. Electrospray ionization mass spectroscopy (ESI-MS) was carried out on a Trio 2000 instrument (VG Biotech, Altrincham, UK).

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[1] The abbreviations used are: Met-RANTES, methionylated RANTES; HPLC, high performance liquid chromatography; p_L, λ leftwards promoter; MAP, methionine amino peptidase.

Samples from reverse phase HPLC (trifluoroacetic acid/acetonitrile system) were dried and then dissolved in methanol/water/acetic acid (49.5/49.5/1 (v/v)) and infused at a rate of 2 ml/min. Spectra were co-added by repetitively scanning until an acceptable signal to noise ratio was obtained (3–4 min). Circular dichroism spectra were obtained using a Jasco J600 spectropolarimeter. ^{1}H NMR spectroscopy was according to Chung *et al.* (13).

Bioassays—THP-1 cell chemotaxis was carried out using 96-well micro-Boyden chambers (Neuro-Probe, Cabin John, MD) fitted with 5-μm filters. 5.6×10^5 cells in 200 μl of medium (RPMI 1640 containing 0.01 M HEPES, 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, and 0.005% gentamicin) were placed in the upper chamber. 370 μl of the medium described above, but without fetal calf serum, containing the ligand and appropriate dilutions of Met-RANTES, were placed in the lower chamber. After 60 min of incubation at 37 °C under 5% CO_2, the cells were removed from the upper wells, and 200 μl of phosphate-buffered saline containing 20 μM EDTA added to detach the cells bound to the filter. After 30 min of incubation at 4 °C, the plate was centrifuged at 1800 × *g* for 10 min, and the supernatants were removed from the lower wells. The number of cells that had migrated were measured by the Cell Titer 96® nonradioactive cell proliferation assay (Promega), which monitors the conversion of tetrazolium blue into its formazan product. T cell chemotaxis was performed according to Fincham *et al.* (14), and calcium mobilization was performed in THP-1 cells according to Tsien *et al.* (15) with the modifications described previously (16). The data obtained were fitted using Grafit 3.01 software (17) to a four-parameter logistic equation. The IC_{50} is defined as the concentration of antagonist giving half the maximal inhibition. Typical agonist concentrations were 5× the EC_{50} value determined for induction of the response.

Recombinant Receptor Expression—MIP-1α/RANTES (CC CKR1) receptor was cloned by reverse transcriptase PCR from the human eosinophilic cell line EOL-3 using specific primers based on the published cDNA sequence (9). CC-CKR-1 cDNA was subcloned into the mammalian cell expression vector pcDNA1 neo (Invitrogen) and stably expressed in the HEK 293 cell line as described elsewhere (12).

Receptor Binding Assays—Equilibrium competition binding assays were carried out according to Van Riper *et al.* (18) but including 0.02% sodium azide in the binding buffers. The data obtained were fitted using Grafit (17) into the equation, $B = B_{max}[L]/(K_d + [L])$.

Enzymic Digestion—0.27 ml of the leucine aminopeptidase ammonium sulfate suspension (Sigma) was brought into solution by adding 1.15 ml of 38 mM sodium phosphate buffer, pH 7.2, containing 9.5 mM $MgCl_2$, 1.9 mM phenylmethylsulfonyl fluoride, and 1.9 mM benzamidine-HCl. The solution was centrifugally concentrated in a Centricon 10 cell to 0.35 ml, a further 1.15 ml of buffer-inhibitor solution was added, and the volume was again reduced to 0.35 ml. 0.8 mg of Met-RANTES was dissolved in H_2O and adjusted to 2 mg/ml in a phosphate-buffered saline solution (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 14 mM NaCl, 0.3 mM KCl, pH 7.2), containing 0.45 M sodium phosphate buffer, pH 7.2, 5 mM $MgCl_2$, 2.4 mM phenylmethylsulfonyl fluoride, and 2.4 mM benzamidine-HCl. 0.08 mg of the leucine amino peptidase solution described above was added, and the solution was adjusted to 0.1% sodium lauryl sarcosinate. Incubation was carried out at 23 °C, and samples were removed at the times indicated in Fig. 1. The amount of methionine removed was analyzed by isocratic elution on reverse phase HPLC.

RESULTS

Protein Characterization—Very high levels of recombinant RANTES were obtained when the cDNA coding for the mature form of the protein was expressed under control of the T7 RNA polymerase system, although the protein would not express in *E. coli* when driven by either the p_L or the Trp promoter. The protein was purified from inclusion body material, renatured, and shown to be refolded by both NMR (13) and CD spectroscopy (results not shown). Electrospray ionization mass spectroscopy analysis of the purified protein gave a mass of 7,978 as opposed to the expected mass of 7,847 for the oxidized mature protein. Amino terminal sequence analysis showed that the initiating methionine had been retained, accounting for the additional mass of 131. We have therefore called the protein Met-RANTES. Unlike naturally isolated (19) or full-length recombinant RANTES (2), Met-RANTES was unable to cause chemotaxis or calcium mobilization in the promonocytic cell line THP-1, even when using concentrations as high as 10^{-6} M.

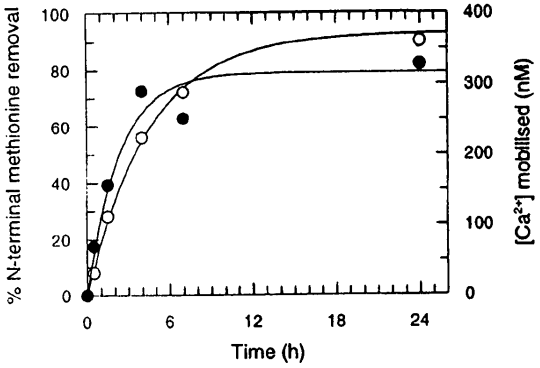

FIG. 1. **Incubation of Met-RANTES with leucine aminopeptidase produces a protein that can mobilize a calcium signal in THP-1 cells.** The appearance of activity (●) correlates with the removal of the amino-terminal methionine residue (○) 0.8 mg of Met-RANTES was incubated with 0.08 mg of leucine aminopeptidase as described in the text.

Comparison of the NMR spectra of the methionylated and nonmethionylated forms of RANTES showed that no major conformational change had been induced by addition of the amino-terminal amino acid. In both cases, the three-dimensional location of the amino terminus of RANTES is partially disordered in solution, similar to the results described for another CC chemokine MIP-1β (20). Since the only difference between active RANTES and our Met-RANTES was the initiating methionine, we postulated that this residue was responsible for the observed difference in activity between the two proteins. This was tested by treating Met-RANTES with CNBr to remove the methionine. Activity was observed even without a further renaturation following the harsh treatment in 70% formic acid. Enzymic removal of the amino-terminal methionine using leucine aminopeptidase (Fig. 1) also activated the protein. These results further confirm that the initial lack of activity in Met-RANTES is not due to misfolding of the recombinant methionylated protein.

Antagonistic Properties—Since it was correctly folded, we next attempted to antagonize the function of RANTES in chemotaxis assays using the methionylated protein (Fig. 2*a*). A concentration of 3.5 nM RANTES was used as the chemotactic stimulus for THP-1 cells, 5 times the EC_{50} value for RANTES in this assay. The Met-RANTES clearly inhibits the chemotactic response under these conditions, with an IC_{50} value of 6 nM, which is a 1.7-fold molar excess. Since RANTES and MIP-1α share a common receptor, (9, 10), we also studied the effect of Met-RANTES against MIP-1α induced chemotaxis of THP-1 cells. Again, nanomolar inhibition was seen, with an IC_{50} value of 0.49 nM, using 1 nM MIP-1α (5 × EC_{50}) as a stimulus (Fig. 2*a*). RANTES also induces migration of T cells (2), and Met-RANTES was able to antagonize the response to 23 nM RANTES (5 × EC_{50}) with an IC_{50} of 6 nM (Fig. 2*b*), which is the same concentration as the EC_{50} for RANTES-induced response. As with the THP-1 cells, Met-RANTES antagonized the response induced by 0.5 nM MIP-1α (5 × EC_{50}), with an IC_{50} of 6 nM, which is 60-fold higher than the EC_{50} for MIP-1α-induced lymphocyte chemotaxis (Fig. 2*b*). No antagonism of MCP-1-induced chemotaxis of THP-1 cells or IL-8 induced chemotaxis of T cells was observed, in keeping with the known receptor selectivity of the MIP-1α/RANTES receptor, CC-CKR1 (9, 10).

As a second bioassay, we studied the ability of chemokines to mobilize calcium in THP-1 cells (Fig. 3). Here again, Met-RANTES was capable of antagonizing the effects of RANTES. The concentration required for half-maximal inhibition of the calcium mobilized by 66 nM RANTES (5 × EC_{50}) was 88 nM.

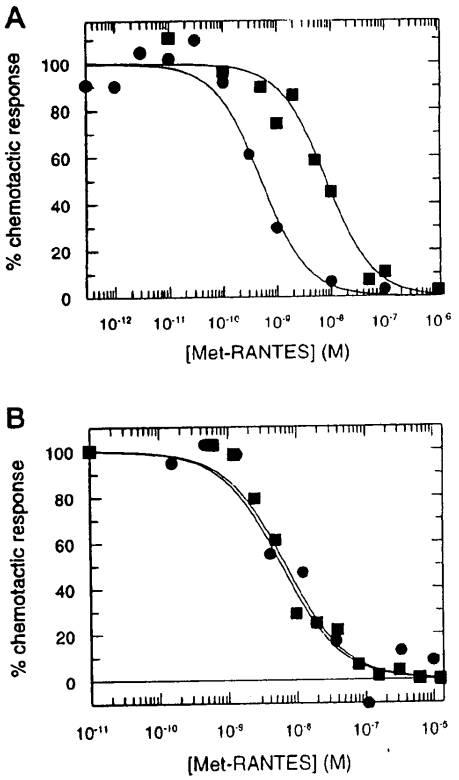

FIG. 2. **Antagonism of chemotaxis by Met-RANTES.** The concentrations of agonist are 5 times higher than the EC_{50} values. Each point shown represents four experiments, in which the measurements were performed in triplicate. The assays were carried out as described in the text in Boyden chamber systems. *a*, chemotaxis of THP-1 cells was induced by adding 3.5 nM RANTES (■) or 1 nM MIP-1α (●) to the lower chamber. The cells migrating to the lower chamber were measured by a viability assay. The maximal chemotactic indices were 11 for RANTES (EC_{50} 0.7 nM) and 5 for MIP-1α (EC_{50} 0.1 nM). *b*, the inhibition of T cell chemotaxis induced by 23 nM RANTES (■), and 0.5 nM MIP-1α (●) added to the lower chamber was measured by the counting the cells on the filter between the upper and lower chambers. The maximal chemotactic indices were 9 for RANTES (EC_{50} 5 nM) and 6 for MIP-1α (EC_{50} 0.2 nM).

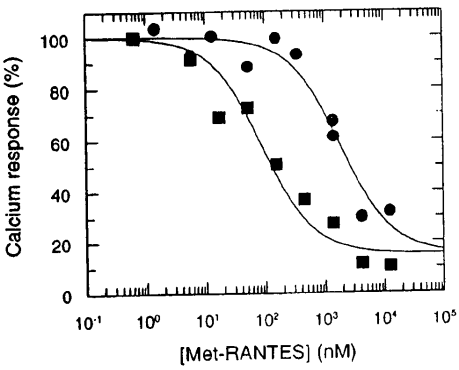

FIG. 3. **Antagonism of the calcium signal in THP-1 cells by Met-RANTES.** The calcium signal was induced by 66 nM RANTES (■) or 23 nM MIP-1α (●), 5 × the EC_{50} values for the maximal calcium mobilization. RANTES induced a maximal response of 375 nM calcium and MIP-1α a maximal response of 260 nM. The results shown are the mean of three experiments.

Although Met-RANTES was capable of inhibiting the mobilization of calcium by 23 nM (5 × EC_{50}) MIP-1α, an inhibitory effect was only seen at concentrations above 100 nM, and the IC_{50} of 1.2 μM is 260-fold higher than the EC_{50} value (4.6 nM) of

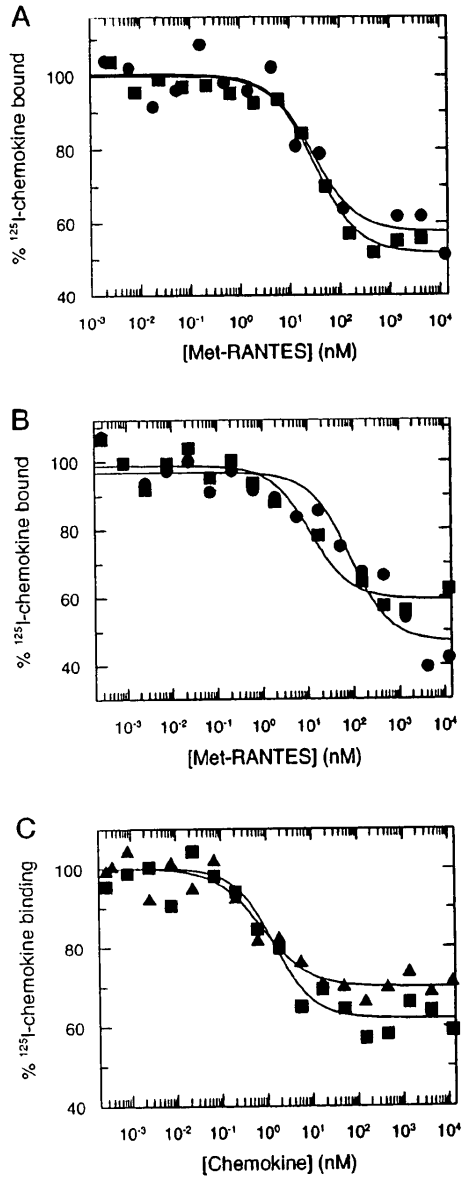

FIG. 4. **Equilibrium binding competition assays.** *a*, displacement of 0.4 nM [^{125}I]RANTES (■) or [^{125}I]MIP-1α (●) from 10^5 THP-1 cells using Met-RANTES. The cells were incubated for 2 h at 4 °C in 100 μl of 50 mM Hepes buffer, pH 7.2, containing 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% BSA, and 0.002% sodium azide, and then washed 4 times with ice-cold buffer containing 0.5 M NaCl using a vacuum pump. *b*, displacement of 0.4 nM [^{125}I]RANTES (■) or [^{125}I]MIP-1α (●) from the MIP-1α/RANTES receptor, also known as CC-CKR 1 stably expressed in HEK 293 cells was carried out as described above. *c*, displacement of 0.4 nM [^{125}I]Met-RANTES ( ) and 0.4 nM [^{125}I]RANTES (■) by RANTES from the CC-CKR 1 receptor in HEK 293 cells as described above.

the agonist-induced response. It was again unable to antagonize the calcium mobilization produced as a result of stimulation with either MCP-1 or IL-8, which are known to act at distinct receptors (21–23)

Receptor Binding—Equilibrium competition binding studies using the promonocytic cell line, THP-1 (Fig. 4*a*) showed that Met-RANTES could effectively compete for both [^{125}I]RANTES and [^{125}I]MIP-1α. Met-RANTES competes for the binding of [^{125}I]RANTES with an IC_{50} of 25 nM, and for [^{125}I]MIP-1α binding with an IC_{50} of 28 nM.

A shared receptor for RANTES and MIP-1α has been iden-

tified (9). We therefore tested the ability of Met-RANTES to compete with [^{125}I]RANTES and [^{125}I]MIP 1α on HEK 293 cells stably transfected with the CC-CKR 1 receptor (Fig. 4*b*). The IC_{50} values are similar to those obtained on the THP-1 cells. Met-RANTES competed for binding of [^{125}I]RANTES with an IC_{50} of 9 nM and for [^{125}I]MIP 1α with an IC_{50} of 84 nM. In order to demonstrate that the Met-RANTES could bind directly to this receptor, we tested the ability of RANTES to displace both [^{125}I]RANTES and [^{125}I]Met-RANTES. RANTES competes for the binding of [^{125}I]RANTES with an IC_{50} of 0.6 nM and for [^{125}I]Met-RANTES with an IC_{50} of 0.8 nM (Fig. 4*c*).

DISCUSSION

The retention of the initiating methionine in recombinant proteins expressed heterologously in *E. coli*, particularly those produced in inclusion bodies, is not uncommon. This is not always the case, as the endogenous *E. coli* MAP (24) often removes the Met residue. The retention of the initiating Met does not impair bioactivity in the case of recombinant cytokines such as granulocyte-macrophage colony stimulating factor (25), interleukin-2 (26), and interleukin-5 (27). However some 5–10-fold shifts in receptor binding affinity have been seen in certain cases such as hirudin (28) and interleukin-1β (29).

The retention of methionine in recombinant RANTES produces a protein that shows no agonist activity, despite the fact that it is correctly folded (13). Furthermore, it acts as a functional antagonist. The addition of a single amino acid to the amino terminus of RANTES creates an antagonist that is almost equipotent: a 1.7-fold molar excess is required for half-maximal inhibition of RANTES-induced chemotaxis of THP-1 cells, whereas the RANTES-induced T cell chemotaxis is inhibited with an IC_{50} equal to the EC_{50} for the response. Similarly, the calcium mobilization induced by RANTES is inhibited to 50% with an equimolar concentration to that used to induce the response. The same potency is observed for the inhibition of chemotaxis induced by MIP-1α, both in THP-1 and T cells. Interestingly, however, the IC_{50} value for the inhibition of the MIP-1α induced calcium mobilization in THP-1 cells is 260-fold higher than the EC_{50}. Met-RANTES therefore antagonizes RANTES and MIP-1α with similar potency in the chemotaxis response but shows a clear difference in the other assay, calcium mobilization. This suggests that either there is an additional receptor for these chemokines or that there are two different signaling pathways for these two responses. A third possibility would be that different ligands evoke distinct signaling pathways at the same receptor.

The extension of RANTES by a single amino acid at the amino terminus thus produces a more potent antagonist than those described for other chemokines, which have been produced by amino-terminal deletions. Data from mutagenesis studies of the CXC chemokine interleukin-8 shows that the amino-terminal region is crucial for signaling, and deletion of five amino acids produces an antagonist (35, 36). However, a 30-fold molar excess is required for half-maximal inhibition of elastase release, and the reduction of neutrophil chemotaxis is not complete at 10^{-6} M (36). Residues 9–76 of the CC chemokine, monocyte chemotractant protein-1 (MCP-1) produce an antagonist that inhibits THP-1 cell chemotaxis with an IC_{50} of 20 nM induced by 5 nM MCP-1 (37). Truncation of residues 2–8 from the amino terminus of MCP-1 produces an antagonist (38), which causes half-maximal inhibition of monocyte chemotaxis at a ratio of 75:1. This variant has been suggested to act as a dominant negative repressor of the active form of the ligand (39). However, the Met-RANTES antagonist appears to act as a competitive inhibitor on the shared MIP-1α/RANTES receptor since, first, direct binding of [^{125}I]Met-RANTES has been demonstrated, and second, it can compete with both chemokines for binding.

Single amino acid changes have produced antagonists of other cytokines, such as interleukin-4 (32), interleukin-6 (33), and granulocyte-macrophage colony-stimulating factor (34). In the cases of these cytokines, they bind and signal through heterodimeric receptor complexes. Detailed studies have shown that the side chains involved in the initial binding between the ligand and its receptor, and the side chains involved in causing a signaling response are spatially separated. A mutant becomes an antagonist if its region interacting with the signaling part of the receptor can be eliminated without altering the binding region. To date, no mutagenesis data is available on RANTES, indicating whether the residues involved in binding and signaling differ. Chemokines, and other chemotactic peptides such as C5a, bind and signal through a seven-transmembrane G-protein coupled receptor. C5a interacts with its receptor through two distinct sites (30). Binding of C5a to the amino terminus of the receptor is proposed to produce a conformational change in the ligand, allowing it to properly interact with the second site at the carboxyl terminus to achieve functional activation (31). Such a dramatic change in bioactivity by the addition of a single amino acid emphasizes the importance of the amino terminus of RANTES. The extension of the sequence by one residue has removed the ability to form a functional interaction with the region of the receptor involved in G-protein-mediated signal transduction. However, since the overall structure of the protein is unaffected, receptor binding is unimpaired, producing a highly potent antagonist. *In vivo* studies with this readily available molecule will be extremely valuable in determining whether blocking the RANTES receptor(s) involved in the recruitment of leucocytes to inflammatory sites will relieve chronic inflammation.

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REFERENCES

1. Oppenheim, J. J., Zachariae, C. O. C., Mukaida, N., and Matsushima, K. (1991) *Annu. Rev. Immunol.* **9**, 617–648
2. Schall, T. J., Bacon, K., Toy, K. J., and Goeddel, D. V. (1990) *Nature* **347**, 669–671
3. Beck, L. A. (1994) *J. Allergy. Clin. Immunol.* **93**, 234
4. Meurer, R., Van Riper, G., Feeney, W., Cunningham, P., Hora, D., Springer, M. S., MacIntyre, D. E., and Rosen, H. (1993) *J. Exp. Med.* **178**, 1913–1921
5. Murphy, W. J., Taub, D. D., Anver, M., Conlon, K., Oppenheim, J. J., Kelvin, D. J., and Longo, D. L. (1994) *Eur. J. Immunol.* **24**, 1823–1827
6. Rot, A., Krieger, M., Brunner, T., Bischoff, S. C., Schall, T. J., and Dahinden, C. A. (1992) *J. Exp. Med.* **176**, 1489–1495
7. Bischoff, S. C., Krieger, M., Brunner, T., Rot, A., Tscharner, V. V., Baggiolini, M., and Dahinden, C. A. (1993) *Eur. J. Immunol.* **23**, 761–767
8. Alam, R., Stafford, S., Forsythe, P., Harrison, R., Faubio, D., Lett-Brown, M. A., and Grant, J. A. (1993) *J. Immunol.* **150**, 3442–3447
9. Neote, K., DiGregorio, D., Mak, J. Y., Horuk, R., and Schall, T. J. (1993) *Cell* **72**, 415–425
10. Gao, J.-L., Kuhns, D. B., Tiffany, H. L., McDermott, D., Li, X., Francke, U., and Murphy, P. M. (1993) *J. Exp. Med.* **199**, 1421–1427
11. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Methods Enzymol.* **185**, 60–89
12. Proudfoot, A. E. I., Power, C. A., Hoogewerf, A. J., Montjovent, M.-O., Borlat, F., and Wells, T. N. C. (1995) *FEBS Lett.* **376**, 19–23
13. Chung, C., Cooke, R., Proudfoot, A. E. I., and Wells, T. N. C. *Biochemistry*, in press
14. Fincham, N. J., Camp, R. D. R., Gearing, A. J. H., Bird, C. R., and Cunningham, F. M. (1988) *J. Immunol.* **140**, 4294–4299
15. Tsien, R. Y., Pozzan, T., and Rink, T. J. (1982) *J. Cell Biol.* **94**, 325–334
16. Lusti-Narasimhan, M., Power, C. A., Allet, B., Alouani, S., Bacon, K. B., Mermod, J.-J., Proudfoot, A. E. I., and Wells, T. N. C. (1995) *J. Biol. Chem.* **270**, 2716–2721
17. Leatherbarrow, R. J. (1993) *Grafit*, Version 3.01, Erithicus Software, Ltd., Staines, UK
18. Van Riper, G., Siciliano, S., Fischer, P. A., Meurer, R., Springer, M. S., and Rosen, H. (1993) *J. Exp. Med.* **177**, 851–856
19. Kameyoshi, Y., Dörschner, A., Mallet, A. I., Christophers, E., and Schröder, J-M. (1992) *J. Exp. Med.* **176**, 587–592
20. Lodi, P. J., Garrett, D. S., Kuszewski, J., Tsang, M. L., Weatherbee, J. A., Leonard, W. J., Gronenborne, A. M., and Clore, G. M. (1994) *Science* **263**, 1762–1767
21. Charo, I. F., Myers, S. J., Herman, A., Franci, C., Connolly, A. J., and

Coughlin, S. R. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 2752–2756
22. Holmes, M. E., Lee, J., Kuang, W. J., Rice, G. C., and Wood, W. I. (1991) *Science* **253**, 1278–1280
23. Murphy, P. M., and Tiffany, H. L. (1991) *Science* 253, 1280–1282
24. Hirel, Ph.-H., Schmitter, J.-M., Dessen, P., Fayat, and G. Blanquet, S. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 8247–8251
25. DeLamarter, J. F., Mermod, J.-J., Liang, C. M., Eliason, J. F., and Thatcher, D. R. (1985) *EMBO J.* **4**, 2575–2581
26. Liang, S. M., Allet, B., Rose, K., Hirschi, M., Liang, C. M., and Thatcher, D. R. (1985) *Biochem. J.* **229**, 429–439
27. Proudfoot, A. E. I., Fattah, D., Kawashima, E. H., Bernard, A., and Wingfield, P. T. (1990) *Biochem. J.* **270**, 357–361
28. Loison, G. (1988) *Bio/Technology* **6**, 72–77
29. Wingfield, P. T., Graber, P., Movva, N. R., Groenenborn, A. M., and MacDonald, H. R. (1987) *FEBS Lett* **215**, 160–164
30. Siciliano, S. J., Rollins, T. E., DeMartino, J., Konteatis, Z., Malkowitz, L., Van Riper, G., Bondy, S., Rosen, H., and Springer, M. S. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 1214–1218
31. DeMartino, J. A., Van Riper, G., Siciliano, S. J., Molineaux, C. J., Konteatis, Z. D., Rosen, H., and Springer, M. S. (1994) *J. Biol. Chem.* **269**, 14446–14450
32. Kruse, N., Tony, H.-P., and Sebald, W. (1992) *EMBO J.* **11**, 3237–3244
33. Brakenhoff, J. P. J., de Hon, F. D., Fontaine, V., ten Boekel, E., Schooltink, H., Rose-John, S., Heinrich, P. C., Content, J., and Aarden, L. A. (1994) *J. Biol. Chem.* **269**, 86–93
34. Hercus, T. J., Bagley, C. J., Cambareri, B., Dottore, M., Woodcock, J. M., Vadas, M. A., Shannon, M. F., and Lopez, A. F. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 5838–5842
35. Hébert, C. A., Vitangcol, R. V., and Baker, J. B. (1991) *J. Biol. Chem.* **266**, 18989–18994
36. Moser, B., Dewald, B., Barella, L., Schumacher, C., Baggiolini, M., and Clark-Lewis, I. (1993) *J. Biol. Chem.* **268**, 7125–7128
37. Zhang, Y. J., Rutledge, B. J., and Rollins, B. J. (1994) *J. Biol. Chem.* **269**, 15918–15924
38. Gong, J. H., and Clark-Lewis, I. (1995) *J. Exp. Med.* **181**, 631–640
39. Zhang, Y. J., and Rollins, B. J. (1995) *Moll. Cell. Biol.* **15**, 4851–4855